Accuwell 17-alpha-Hydroxyprogesterone EIA 510(k) Summary

K060452

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2) Device Trade or Proprietary Name: Accuwell 17-a-hydroxyprogesterone (OHP) enzyme immunoassay (EIA)

Device Common or Usual Name: 17-a-hydroxyprogesterone (OHP) neonatal screening enzyme immunoassay (EIA)

Device Classification Name: Radioimmunoassay, 17-hydroxyprogesterone (JLX)

3) Legally Marketed Predicate Device: AutoDELFIA Neonatal 17a-OH-progesterone, Model B048-112

4) Device Description:

Summary and Explanation of the Assay

Congenital Adrenal Hyperplasia (CAH) is a family of genetic disorders caused by reduced enzyme activities, which are required in the biosynthesis pathway leading to the production of cortisol in the adrenal cortex. The most common of these disorders is the 21-hydroxylase deficiency and accounts for more than 90% of CAH cases. [1, 2, 3]

The incidence of classical 21-hydroxylase deficiencies varies widely by population and ethnic group. In Japan the incidence is thought to be about 1 in 21,000, while in Europe and North America incidence ranges between 1 in 10,000 to 1 in 16,000. The Yupik Eskimos in Alaska have an extremely high incidence of around 1 in 300.[4,5]

Clinical symptoms of the 21-hydroxylase deficiency occur primarily due to the over production of precursors to the blocked enzymatic step. These precursors are shunted into the androgen biosynthesis pathway, which produces virilization in the female fetus, rapid postnatal growth with accelerated skeletal maturation, precocious puberty and short stature in both males and females.[6] About 75% of the patients with classical CAH also have a defect in their ability to synthesize aldosterone, which can lead to a salt wasting crisis.

In classical 21-hydroxylase deficiencies the 17 α-hydroxyprogesterone (17-OHP) levels are markedly increased. However, the 17-OHP levels in normal infants are elevated in the first two to three days after birth. These high concentrations in normal infants decline rapidly to adult levels within the first seven to ten days.[7] Low birth-weight, premature and sick babies may continue to have higher than normal 17-OHP levels for an extended time frame. For this reason it has been recommended that a multi-tiered approach

be employed to setting the normal cut-off. Laboratories must be notified of either dexamethasone therapy or transfusion status. Due to the population differences it is also strongly recommended that each laboratory establish their own normal range and cutoff levels. Periodic review and possible adjustment if necessary to reduce the false positive rate is also recommended. Non-classical forms of CAH are not reliably detected by newborn screening.[8, 9, 10, 11]

The Working Group on Neonatal Screening of the European Society of Pediatric Endocrinology (ESPE) has recommended that after a positive screening test for CAH, it is imperative that the diagnosis be validated with confirmatory testing. Confirmation methods vary depending on availability but include urine steroid analysis, ACTH stimulation and CY21 gene analysis.[12, 13, 14, 15]

Principle of the Assay

The Accuwell™ 17α-Hydroxyprogesterone EIA Kit is a competitive solid phase enzyme immunoassay (EIA) for the quantitative measurement of 17α-Hydroxyprogesterone (17-OHP) concentrations in neonatal blood samples that have been collected onto Whatman S&S® 903™ specimen collection paper. Standard control, and unknown dried blood spot sample discs are added to specified wells in a 96-well microplate coated with antiserum specific for 17-OHP.

Two procedures are presented.

The first procedure includes an elution step where blood is eluted from the dried blood spot sample. A 1/8 inch disc from standards, controls, and unknown dried blood spot samples are added to specified wells in an uncoated microplate. Saline is added to all wells and the plate is allowed to incubate until the blood has eluted from the paper. Immediately following a transfer of a portion of this eluate into the antibody-coated microplate a horseradish peroxidase enzyme conjugated to 17-OHP (17-OHP: HRP) is added to the wells. A competition begins between the endogenous 17-OHP from the unknown samples, standards and controls and the 17-OHP: HRP for the limited number of antibody binding sites in the wells. An inverse relationship develops between the concentration of endogenous 17-OHP and the amount of 17-OHP: HRP, which will bind to the coated microplate. After a period of incubation the excess 17-OHP: HRP is removed by washing. A color developer, containing a colorless 3, 3', 5, 5'tetramethylbenzidine (TMB) and peroxide (H_2O_2), is added to all wells. The peroxide and peroxidase react and subsequently the TMB is converted from colorless to a blue color. The color development is terminated through the addition of a stopping reagent and the absorbance is measured at 650 nm using a microplate absorbance reader. A standard curve is constructed using the known concentration of each standard plotted against the corresponding absorbance reading of that standard. The concentrations of unknown controls and samples are determined by comparison to this standard curve.

An alternate procedure is described where a 1/8 inch disc from each blood spot is placed directly into designated wells of the coated microplate. The enzyme conjugated 17-OHP is added and the competition proceeds concurrent with the elution step. Upon completion of this incubation period, the excess 17-OHP:HRP and the discs are removed by decanting, or aspirating the discs from each well. The plate is then washed and processed using the same procedure as described above.

Kit Contents

Materials Supplied

	Quantity per Kit		
Wells	96	384	1920
Catalog No.	601501	601502	601503
Coated Microplates (Rabbit Anti-17-OHP)	1 Strip plate	4 plates	20 plates (5 bags of 4 plates each)
Enzyme Conjugate Concentrate (17-OHP:HRP)*	2 mls	5 mls	25 mls
Conjugate Diluent*	20 mls	50 mls	250 mls

Wash Buffer Concentrate (20X)	25 mls	50 mls	250 mls
Color Developer *	20 mls	55 mls	250 mls
Stopping Reagent	20 mls	55 mls	250 mls
Multi-Analyte Standards and Controls TSH/T4/17-OHP	1 set	1 set	4 sets

*These reagents are light sensitive. Avoid prolonged exposure to light.

NOTE: Do not use reagents or solutions that have become cloudy or discolored as these conditions indicate deterioration. The reagents should be stored in their original containers.

Do not exchange reagents from one kit with those of another kit unless the lot number and expiration of the reagent are the same.

Reagent Description

Coated Microplates (Rabbit Anti-17-OHP)
Microplates are coated with an antiserum produced in rabbits immunized with a 17-OHP hapten. The microplates are packaged in zipper-lock foil bags containing a desiccant. Store the unused microplates and/or strips from the strip plate in the zipper-lock bags with desiccant. Each microplate is labeled with a unique bar-code label.
Storage: Dry at 2-25° C
Expiration: Refer to the expiration date printed on the label

Enzyme Conjugate Concentrate (17-OHP: HRP)*
A 17-OHP derivative conjugated to horseradish peroxidase, danazol with an enzyme stabilizer.
*This reagent is light sensitive, plate store in the original brown container.
Enzyme Conjugate Concentrate must be diluted with Conjugate Diluent before use.
See Reagent Preparation Instructions section on page 11.
Storage: 2-8° C Protected From Light.
Expiration: Refer to the expiration date printed on the label.

Conjugate Diluent*
Tris-buffer, bovine serum and preservatives. *This reagent is light sensitive, store in the original brown container. Conjugate Diluent is used to dilute the conjugate concentrate before use.
See Reagent Preparation Instructions section on page 11.
Storage: 2-8° C Protected from Light.
Expiration: Refer to the expiration date printed on the label.

Wash Buffer Concentrate (20X)
A concentrated solution of phosphate buffered saline containing a surfactant.
Wash Buffer Concentrate must be diluted with deionized or distilled water before use.
See Reagent Preparation Instructions section on page 11.
Storage: 2-25° C
Expiration: Concentrate: Refer to the expiration date printed on the label.
Diluted wash buffer is stable for 1 month when stored at 2-25° C.

Color Developer*
Bottle contains a colorless solution of 3, 3′, 5, 5′-Tetramethylbenzidine in a diluted organic solvent with citrate buffer and hydrogen peroxide. *This reagent is light sensitive, store in the original brown container. This reagent should remain colorless; if it has discolored, discard it.
Storage: 2-8° C. Protected from light.
Expiration: Refer to the expiration date printed on the label.

Stopping Reagent
Bottle contains a dilute solution of sodium fluoride (NaF) and a red dye.
Storage: 2-8° C.
Expiration: Refer to the expiration date printed on the label.

Multi-Analyte Standards and Controls
TSH, T4, 17-OHP
Multi-Analyte standards have been prepared from human whole blood, adjusted to a hematocrit of 55%. Each card of standards contains two circles each of six concentrations of 17-OHP at approximately zero, 10, 25, 50, 100 and 250 ng/ml serum equivalents. Control cards contain four circles each of three different concentrations of 17-OHP at approximately 15, 40, and 90 ng/ml serum equivalent. The standards and controls are spotted onto Whatman (previously known as Schleicher and Schuell (S&S®) 903™) specimen collection paper. Refer to the information on the labels for the exact concentrations of the standards and acceptable ranges for the controls. Each set of standards and controls consists of two standard cards and one control card.

Storage: Dry at 2-8° C or below.
Expiration: Refer to the expiration date printed on the label or Certificate of Analysis received with the kit.

Standard and control units are expressed in ng/ml. The values may be converted using the following formula.

1 nmol/l blood= 0.33 ng/ml blood
1 nmol/l blood= 0.73 ng/ml serum (at 55% hematocrit)

Conversion Table

	Concentration in Serum ng/ml	Concentration in Blood nmol/l
Standard Zero	0	0
Standard A	10	13.7
Standard B	25	34.2
Standard C	50	68.5
Standard D	100	137
Standard E	250	342
Control 1	15	20.6
Control 2	40	54.8
Control 3	90	123

This is an example only. Consult standard and control label or Certificate of Analysis received with the kit for exact concentrations.

Materials Required But Not Supplied for Both the Elution and Direct Procedures

1. 1/8 inch (3 mm) diameter hole punch
2. Forceps or fine tweezers to pick up the punched sample discs
3. Pipettes to accurately dispense 100 µl volumes
4. Multi-channel pipettes to dispense 300 µl volumes or an automated plate washer
5. Microplate reader capable of reading at a wavelength of 650 nm
6. Plate rotator capable of 100-120 RPM or plate shaker.
7. Microplate covers; plate sealers or low evaporation solid plastic microplate lids (e.g. NUNC lids with condensation rings and evaporation barriers)
8. Graduated cylinders
9. Deionized or distilled water

Additional Materials Required for the Elution Procedure Only
10. Un-coated round bottom 96 well microplates
11. Saline (0.85% NaCl).
12. Multi-channel pipet with disposable tips to accurately transfer 15 µl of eluate.

Additional Materials Required for the Direct Procedure Only
13. Aspiration device capable of removing discs from coated plates.

5) Intended Use:
The Accuwell™ 17α-Hydroxyprogesterone EIA Kit is designed for the quantitative measurement of 17α-Hydroxyprogesterone (17-OHP) concentrations in neonatal blood samples that have been collected onto Whatman [previously known as Schleicher and Schuell (S&S®) 903™] specimen collection paper. The results are used to screen newborns for classical congenital adrenal hyperplasia (CAH).

6) Summary of Technological Characteristics [1, 2]:

Characteristic:	**Predicate Device: K 042425; Model B048-112 Wallac Oy AutoDelfia Neonatal 17α-Hydroxyprogesterone Time-resolved flouoroimmunoassay**	**Proposed Device: Neo-Genesis Accuwell 17α-Hydroxyprogesterone EIA**
Intended User	Clinical Laboratory Professionals	Clinical Laboratory Professionals
Intended Use	Measure 17α-Hydroxyprogesterone (17-OHP) levels in neonatal dried blood spots	Measure 17α-Hydroxyprogesterone (17-OHP) levels in neonatal dried blood spots
Indications for Use	Screening for increased levels of 17-OHP in newborns	Screening for increased levels of 17-OHP in newborns
Chemical Principle	Competitive reaction between europium-labeled 17-OHP and sample 17-OHP for a limited number of binding sites on 17-OHP specific polyclonal antibodies derived from rabbit	Competitive reaction between horseradish peroxidase (HRP) labeled 17-OHP and sample 17-OHP for a limited number of binding sites on 17-OHP specific polyclonal antibody derived from rabbit
Assay Type	Time-resolved fluoroimmunoassay	Enzyme-immunoassay
Detection Method	Europium ions dissociated from the labeled antiserum form highly fluorescent chelates with components of an enhancement solution. Fluorescence in each well is then measured	Peroxidase which remains bound to micro-wells reacts with peroxide and TMB subsequently converting the TMB from colorless to a blue color which is measured
Assay Processing Method	Automated	Manual
Detection Equipment	Autodelfia – time resolved fluorometer	Microplate Spectrophotometer
Sample Requirements	Newborn blood collected on S&S 903 filter paper or equivalent collection cards	Newborn blood collected on S&S 903 filter paper

Specimen Rejection Criteria	Sample spot not uniformly saturated with blood; sample spots punched to close to the edge of the blood spot; poorly collected and improperly dried specimens; non-eluting blood spot; contamination of blood spot filter paper with foreign material	Sample spot not uniformly saturated with blood; sample spots punched to close to the edge of the blood spot; poorly collected and improperly dried specimens
Specimen	1/8 inch spot (3.2mm) punched from a standard collection card	1/8 inch spot (3.2mm) punched from a standard collection card of S&S 903 paper
Standard Configuration	Human blood with a hematocrit of 50-55% and spotted on S&S 903 filter paper. Calibrated using gravimetric methods. Seven levels	Human whole blood adjusted to 55% hematocrit and spotted on S&S 903 filter paper. Calibrated using gravimetric methods. Six levels
Standard Range	0 – 190 ng/ml approximate serum equivalent	0 – 250 ng/ml approximate serum equivalent
Control Configuration	Human blood with a hematocrit of 50-55% and spotted on S&S 903 filter paper. Calibrated using gravimetric methods. Two levels	Human whole blood adjusted to 55% hematocrit and spotted on S&S 903 filter paper. Calibrated using gravimetric methods. Three levels
Control Levels	14 and 40 ng/ml approximate serum equivalent	15, 40 and 90 ng/ml approximate serum equivalent
Calculation of Results-Recommendations	Spline Smoothed regression analysis is used to calibrate each plate with the LOG of the concentration on the X-axis and B/Bmax on the Y-axis (response).	Five parameter logistic plot of absorbance versus concentration
Analytical Sensitivity	1.5 ng/ml	2.2 ng/ml
Within Run Precision [3,4]	Mean (ng/ml) 8.8 % cv 11.3 Mean (ng/ml) 29.3 % cv 8.9 Mean (ng/ml) 62.1 % cv 8.9	Mean (ng/ml) 28.2 % cv 3.5 Mean (ng/ml) 56.1 % cv 3.7 Mean (ng/ml) 108.1 % cv 2.7
Between Run Precision [3,4]	Mean (ng/ml) 8.8 % cv 4.0 Mean (ng/ml) 29.3 % cv 3.3 Mean (ng/ml) 62.1 % cv 2.4	Mean (ng/ml) 28.2 % cv 10.3 Mean (ng/ml) 56.2 % cv 8.0 Mean (ng/ml) 108.1 % cv 11.2
Total Precision [3,4]	Mean (ng/ml) 8.8 % cv 12.0 Mean (ng/ml) 29.3 % cv 9.5 Mean (ng/ml) 62.1 % cv 9.2	Mean (ng/ml) 28.1 % cv 11.0 Mean (ng/ml) 56.1 % cv 8.9 Mean (ng/ml) 108.1 % cv 11.6

Interfering Substances	EDTA or citrate blood	Lipemia
Cross Reacting Substances Greater than 0.01 %:		
Progesterone	3.60 %	0.5 %
21-Desoxycortisol	1.75 %	2.42 %
16α-Hydroxyprogesterone	0.3 %	1.2 %
11-Desoxycortisol	0.4 %	0.6 %
11-Deoxy-17-hydroxycorticosterone	0.33 %	< 0.01 %
17α-Hydroxypregnenolone	0.13 %	< 0.01 %
Deoxicorticosterone	0.3 %	0.06 %
5-Pregnen-3β,17-Diol-20-one 3 sulfate	< 0.01 %	0.2 %
Cortisol	< 0.01 %	0.06 %
Tetrahydrocortisone	0.02 %	< 0.01 %
Expected Values- Term (normal birth weight)	3-5 days old, >2000g birthweight (unknown percentile): normal < 22 ng/ml request 2nd sample 22-44 ng/ml inform physician > 44 ng/ml	1-3 days old, ≥ 2500g birthweight (99th percentile): ON Direct: normal < 27.8 ng/ml Follow-up ≥ 27.8 ng/ml ON Eluate: normal < 23.9 ng/ml Follow-up ≥ 23.9 ng/ml 3Hr Eluate: normal < 24.3 ng/ml Follow-up ≥ 24.3 ng/ml ≥ 4 days old, ≥ 2500g birthweight (99th percentile): ON Direct: normal < 21.1 ng/ml Follow-up ≥ 21.1 ng/ml ON Eluate: normal < 19.5 ng/ml Follow-up ≥ 19.5ng/ml 3Hr Eluate: normal < 19.4 ng/ml Follow-up ≥ 19.4 ng/ml
Expected Values- Premature (low birth weight)	3-5 days old, <2000g birthweight (unknown percentile): normal < 44 ng/ml request 2nd sample 44-73 ng/ml inform physician > 73 ng/ml	1-3 days old, < 2500g birthweight (99th percentile): ON Direct: normal < 46.4 ng/ml Follow-up ≥ 46.4 ng/ml ON Eluate: normal < 40.9 ng/ml Follow-up ≥ 40.9 ng/ml 3Hr Eluate: normal < 40.5 ng/ml Follow-up ≥ 40.5 ng/ml

Method Comparison	The AutoDelfia Neonatal 17-OHP kit was compared with the DELFIA Neonatal 17-OHP kit using 1579 infant blood spot specimens. Results showed a mean value of 13.3 ng/ml serum for the AutoDelfia and 12.6 ng/ml serum for the predicate DELFIA kit.	The Accuwell 17-OHP was compared to the Autodelfia 17-OHP kit using whole blood spot specimens from the following infant populations: <u>>2500g b.w., 0-1 days old (n=133)-</u> AutoDelfia Mean (ng/ml) =22.6 Accuwell Means (ng/ml): ON Direct = 16.5 ON Eluate = 16.7 3Hr Eluate = 17.3 <u>>2500g b.w., 2-3 days old(n=133)-</u> AutoDelfia Mean (ng/ml) =16.2 Accuwell Means (ng/ml): ON Direct = 11.3 ON Eluate = 12.2 3Hr Eluate = 12.8 <u>>2500g b.w., >4 days old (n=197)-</u> AutoDelfia Mean (ng/ml) =9.5 Accuwell Means (ng/ml): ON Direct = 7.3 ON Eluate = 7.8 3Hr Eluate = 8.3 <u>1400-2500g b.w., 1-6 days (n=30)-</u> AutoDelfia Mean (ng/ml) =25.5 Accuwell Means (ng/ml): ON Direct = 19.3 ON Eluate = 19.2 3Hr Eluate = 19.3

CDC Control Results [7]				
CDC Lot 451 – Enriched with 25 ng/ml serum	Mean (ng/ml)	30.0	Mean (ng/ml)	28.1
	% cv	11.3	% cv	11.0
CDC Lot 452 – Enriched with 50 ng/ml serum	Mean (ng/ml)	59.3	Mean (ng/ml)	56.1
	% cv	10.6	% cv	8.9
CDC Lot 453 – Enriched with 100 ng/ml serum	Mean (ng/ml)	120.2	Mean (ng/ml)	108.1
	% cv	10.1	% cv	11.6
Intercept =	- 0.45		+ 2.1	
Slope =	1.2049		1.0629	

[1] Data displayed for the predicate device derived from the directions for use unless otherwise noted.

[2] Data displayed for the Accuwell 17-OHP kit was derived using only the overnight eluate method unless otherwise noted.

[3] Results from the predicate device were obtained from their published directions. The method used or definitions were not declared, therefore they may or may not use the same calculation method.

[4] Accuwell 17-OHP precision studies were conducted in accordance with NCCLS EP5-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods"; Approved Guideline-Second Edition. All precision testing was performed by Neo-Genesis at Neo-Genesis' Portland, OR manufacturing facility. Sources of variability included the use of different; operators, days and reagent lot numbers.

[5] Results from an in house study of randomly selected samples from newborns with birth weight >2200 grams. These results are presented as an example only and used strictly as guidance and should not be used to establish reference ranges.

[6] Results from an in house study of randomly selected samples from newborns with birth weight ≤2200 grams. These results are presented as an example only and used strictly as guidance and should not be used to establish reference ranges.

[7] Predicate kit results for 17-OHP were derived from CDC publication "Newborn Screening Quality Assurance Program 2005 Annual Summary Report, Vol. 23, Jan 2006. % CV was calculated using the reported mean and the average within laboratory standard deviation. Intercept and slope are as reported.

7) Clinical and Non-Clinical Data:

Precision

Precision studies were conducted in accordance with NCCLS EP5-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods"; Approved Guideline-Second Edition.

Testing included one run per day, with two sample aliquots per run, performed on 20 different days using each of three different assay procedures. The three procedures were designated as: 1) "ON Eluate", 2) "3 Hr Eluate" and 3) "ON Direct DBS", respectively. The resulting data were used to estimate repeatability, between-day and within-device precision as described by the standard, for each procedure.

All precision testing was performed by Neo-Genesis at Neo-Genesis' Portland, OR manufacturing facility. Sources of variability included the use of different: operators, days and reagent lot numbers.

Summary Results and Estimates of Precision for Accuwell 17-OHP EIA Kit

		Sample ID:		
		CDC 451* (25+ ng/ml)	CDC 452 (50+ ng/ml)	CDC 453 (100+ ng/ml)
ON Eluate	Count	40	40	40
	Mean	28.2	56.1	108.1
	SD	3.0	4.9	12.3
	%CV	10.7	8.8	11.4
Within-Run Standard Deviation:	Sr	1.0	2.1	2.9
Between-Day Standard Deviation:	Sdd	2.9	4.5	12.1
Within Device Standard Deviation:	ST	3.1	5.0	12.5
3 HR Eluate	Count	40	40	40
	Mean	26.2	53.0	104.6
	SD	3.4	4.1	11.9
	%CV	12.9	7.7	11.3
Within-Run Standard Deviation:	Sr	1.9	2.2	5.4
Between-Day Standard Deviation:	Sdd	2.9	3.4	10.7
Within Device Standard Deviation:	ST	3.4	4.1	12.0
ON Direct DBS	Count	40	40	40
	Mean	29.5	57.9	108.6
	SD	4.2	5.8	13.2
	%CV	14.1	10.0	12.2
Within-Run Standard Deviation:	Sr	1.5	4.4	11.7
Between-Day Standard Deviation:	Sdd	3.9	3.9	6.2
Within Device Standard Deviation:	ST	4.2	5.8	13.3

*Enriched as reported by Centers for Disease Control and Prevention. Newborn Screening Quality Assurance Program Annual Report 2005

Analytical Sensitivity

The analytical sensitivity was determined for each procedure including Overnight Eluate, 3 hour Eluate, and the Overnight Direct. Sensitivity was defined by the calculated concentration that corresponds to the mean of the absorbance of the zero standard (N=26) minus two times the standard deviation of those same absorbance measurements.

The zero standard was tested multiple time (N=26) in one assay. Testing was performed in each of the two procedures, eluate and direct, and spanning the range of allowable elution times, three hours and overnight for the eluate procedure.

Extrapolation beyond the upper and lower limits of the standard curve or beyond the limits of detection is not an acceptable laboratory practice. Therefore any sample result falling below these calculated concentrations should be reported as less than the limit of detection.

This data is provided for example only and should be confirmed by each laboratory and appropriate concentrations defined.

	Overnight Eluate	3 Hour Eluate	Overnight Direct
Absorbance Mean	2.506	1.648	2.604
Standard Deviation	0.04	0.02	0.06
Absorbance -2 SD	2.426	1.608	2.484
Calculated Concentration (ng/ml)	2.2	1.5	2.4

Linearity and Recovery

The Accuwell 17-OHP Kit linear range and the measuring (reportable) range is approximately 2.0 ng/ml to 250 ng/ml based on study data described below, the sensitivity study described elsewhere and limited by the value assigned to the highest standard of the calibration curve.

Assay results obtained outside the measuring (reportable) range should be reported as either "less than" or "greater than" the established low or high limit, respectively, as applicable to the individual result.

Dried blood spots representing a range of sample concentrations were prepared for the study. The sample analyte levels in ng/ml were: 10, 24.5, 39, 68, 97, 126, 155, 184, 213, 242, 271 and 300 (n = 12 concentrations).

Four aliquots of each sample of the range of samples prepared, were tested in each of two runs using each of three different assay procedures. The three procedures were designated as:1) "ON Eluate", 2) "3 Hr Eluate" and 3) "ON Direct DBS", respectively. The mean of the total number of values obtained for each sample (n=8) within each procedure, was compared to the samples expected value. Results of the comparisons are described below.

Results of Assay Linearity and % Recovery Study for Accuwell 17-OHP EIA Kit

		ON Eluate		3 Hr Eluate		ON Direct DBS	
Linear Regression Analysis		$y = 1.0036x - 0.3475$ $R^2 = 0.9994$		$y = 1.1236x - 9.3625$ $R^2 = 0.992$		$y = 1.1362x - 9.5154$ $R^2 = 0.99$	
Sample #	Expected (ng/ml)	Actual (ng/ml)	Recovery (%)	Actual (ng/ml)	Recovery (%)	Actual (ng/ml)	Recovery (%)
1	300	298.1	99.4	354.4	118.1	319.9	106.6
2	271	274.4	101.3	284.4	104.9	324.1	119.6
3	242	241.9	100.0	257.9	106.6	261.6	108.1
4	213	212.3	99.7	221.7	104.1	244.0	114.6
5	184	182.9	99.4	194.1	105.5	185.9	101.0
6	155	157.2	101.4	159.8	103.1	157.5	101.6
7	126	131.7	104.5	127.7	101.3	128.5	102.0
8	97	95.2	98.1	94.2	97.1	92.6	95.5
9	68	66.2	97.4	66.4	97.6	69.9	102.8
10	39	38.2	97.9	37.0	94.9	33.3	85.4
11	24.5	23.0	93.9	23.0	93.9	22.8	93.1
12	10	10.4	104.0	10.3	103.0	10.7	107.0
Average Recovery-Overall			99.7		102.5		103.1
Minimum			93.9		93.9		85.4
Maximum			104.5		118.1		119.6

Specificity

Cross Reactivity

The cross reactivity percentage is determined by dividing the concentration of analyte (17-OHP) at 50% displacement by the concentration of the interferant at 50% displacement from the absorbance corresponding to the zero standard.

Trivial Name	Cross Reactivity	Trivial Name	Cross Reactivity
21-Desoxycortisol	2.4 %	Cholesterol	< 0.01 %
16α-Hydroxyprogestrone	1.2 %	Corticosterone	< 0.01 %
11-Desoxycortisol	0.6%	Cortisol Glucuronide	< 0.01 %
Progesterone	0.5 %	Cortisone	< 0.01 %
5-Pregnen-3β,17-Diol-20-one 3 sulfate	0.2 %	Cortisone 21-Sulphate Sodium Salt	< 0.01 %
Cortisol	0.06 %	Dehydroisoandrosterone	< 0.01 %
Desoxycorticosterone	0.06 %	Dehydroepiandrosterone Sodium Sulphate	< 0.01 %
1-Dehydrotestosterone	< 0.01 %	Dexamethasone	< 0.01 %
5β-Dihydrocortisol	< 0.01 %	Estriol	< 0.01 %
5β-Dihydrocortisone	< 0.01 %	Estrone	< 0.01 %
6β-Hydroxycortisol	< 0.01 %	Prednisolone	< 0.01 %
11-Dehydrocorticosterone	< 0.01 %	Prednisone	< 0.01 %
16α-Hydroxypregnenolone	< 0.01 %	Pregnenolone	< 0.01 %
17α-Estradiol	< 0.01 %	Pregnenolone Sulphate, Sodium Salt	< 0.01 %
17α-Hydroxypregnanolone	< 0.01 %	Spironolactone	< 0.01 %
17β-Estradiol	< 0.01 %	Testosterone	< 0.01 %
20α-Hydroxyprogesterone	< 0.01 %	Tetrahydrocortisol	< 0.01 %
Aldosterone	< 0.01 %	Tetrahydrocorisone	< 0.01 %

Interfering Substances

Interferences due to hemoglobin, conjugated and unconjugated bilirubin, and lipids were studied using methods described in NCCLS (document EP7-A.) as guidance.[27]
Hemoglobin and bilirubin (conjugated and unconjugated) caused no detectable interference.
Significant interference was seen from the lipid solutions added to the Accuwell 17-OHP test.

Method **Comparison**

A retrospective study was conducted to compare the results obtained with the Accuwell 17-OHP EIA to those obtained by a currently marketed neonatal 17-OHP screening device. Test samples were submitted to the study as blinded neonatal dried blood spots collected in sequence under routine screening conditions from a U.S. department of public health laboratory. Original screening results for each sample using the predicate test kit were also obtained from the submitting laboratory for method comparison.

A summary of results of the method comparisons are provided in Tables 9 – 20, below. (Samples interpreted below as "Follow-Up" were not confirmed.)

Table 9: Predicate vs Accuwell O/N Direct
Values at the 99th, 97.5th, and 95th Percentiles for a
Population of Babies **≥2500 gm and 0-1 Days** of Age at Sample Collection

		99th Percentile					97.5th Percentile					95th Percentile		
	Accuwell	Normal	Follow-up			**Accuwell**	Normal	Follow-up			**Accuwell**	Normal	Follow-up	
Predicate		< 27.5	≥ 27.5	Totals:	**Predicate**		< 25.8	≥ 25.8	Totals:	**Predicate**		< 21.8	≥ 21.8	Totals:
Normal	< 43.8 N=130	114	**16**	130	Normal	< 43.8 N=130	112	**18**	130	Normal	< 43.8 N=130	100	**30**	130
Follow-Up	≥ 43.8 N = 3	**0**	3	3	Follow-Up	≥ 43.8 N = 3	**0**	3	3	Follow-Up	≥ 43.8 N = 3	**0**	3	3
	Totals:	114	19	133		Totals:	112	21	133		Totals:	100	33	133

The Accuwell 17-OHP EIA (ON Direct) results for this population were: mean =16.5 ng/ml, with a range of 3.7 to 85.6 ng/ml; while the predicate kit yielded: mean =22.6 ng/ml, with a range of 10.1 to 86.0 ng/ml.

The correlation was found to be: y (Predicate) = 0.970 (Accuwell ON Direct) + 6.569, R = 0.9570

Table 10: Predicate vs Accuwell O/N Eluate
Values at the 99th, 97.5th, and 95th, Percentiles for a
Population of Babies **≥2500 gm and 0-1 Days** of Age at Sample Collection

		99th Percentile					97.5th Percentile					95th Percentile		
	Accuwell	Normal	Follow-up			**Accuwell**	Normal	Follow-up			**Accuwell**	Normal	Follow-up	
Predicate		< 23.9	≥ 23.9	Totals:	**Predicate**		< 22.3	≥ 22.3	Totals:	**Predicate**		< 20.2	≥ 20.2	Totals:
Normal	< 43.8 N=130	106	**24**	130	Normal	< 43.8 N=130	103	**27**	130	Normal	< 43.8 N=130	101	**29**	130
Follow-Up	≥ 43.8 N = 3	**0**	3	3	Follow-Up	≥ 43.8 N = 3	**0**	3	3	Follow-Up	≥ 43.8 N = 3	**0**	3	3
	Totals:	106	27	133		Totals:	103	30	133		Totals:	101	32	133

The Accuwell 17-OHP EIA (ON Eluate) results for this population were: mean =16.7 ng/ml, with a range of 5.3 to 65.6 ng/ml; while the predicate kit yielded: mean =22.6 ng/ml, with a range of 10.1 to 86.0 ng/ml..

The correlation was found to be: y (Predicate) = 1.049 (Accuwell ON Direct) + 5.066, R = 0.9257

Table 11: Predicate vs Accuwell 3 Hour Eluate
Values at the 99th, 97.5th, and 95th Percentiles for a
Population of Babies **≥2500 gm and 0-1 Days** of Age at Sample Collection

		99th Percentile					97.5th Percentile					95th Percentile		
	Accuwell	Normal	Follow-up			**Accuwell**	Normal	Follow-up			**Accuwell**	Normal	Follow-up	
Predicate		< 23.0	≥ 23.0	Totals:	**Predicate**		< 21.7	≥ 21.7	Totals:	**Predicate**		< 19.8	≥ 19.8	Totals:
Normal	< 43.8 N=130	104	**26**	130	Normal	< 43.8 N=130	103	**27**	130	Normal	< 43.8 N=130	99	**31**	130
Follow-Up	≥ 43.8 N = 3	**0**	3	3	Follow-Up	≥ 43.8 N = 3	**0**	3	3	Follow-Up	≥ 43.8 N = 3	**0**	3	3
	Totals:	104	29	133		Totals:	103	30	133		Totals:	99	34	133

The Accuwell 17-OHP EIA (3Hr Eluate) results for this population were: mean =17.3 ng/ml, with a range of 5.1 to 71.1 ng/ml; while the predicate kit yielded: mean =22.6 ng/ml, with a range of 10.1 to 86.0 ng/ml..

The correlation was found to be: y (Predicate) = 0.998 (Accuwell ON Direct) + 5.410, R = 0.9291

Table 12: Predicate vs Accuwell O/N Direct
Values at the 95th Percentile for a
Population of Babies **≥2500 gm and 2-3 Days** of Age at Sample Collection

		95th Percentile		
	Accuwell	Normal	Follow-up	
Predicate		< 18.5	≥ 18.5	Totals:
Normal	< 29.2 N=118	113	5	118
Follow-Up	≥ 29.2 N = 15	0	15	15
	Totals:	113	20	133

The Accuwell 17-OHP EIA (ON Direct) results for this population were: mean =11.3 ng/ml, with a range of 3.4 to 81.1 ng/ml; while the predicate kit yielded: mean =16.2 ng/ml, with a range of 6.9 to 81.0 ng/ml..

The correlation was found to be: y (Predicate) = 1.045 (Accuwell ON Direct) + 4.392, R = 0.9785

Table 13: Predicate vs Accuwell O/N Eluate
Values at the 95th Percentile for a
Population of Babies **≥2500 gm and 2-3 Days** of Age at Sample Collection

		95th Percentile		
	Accuwell	Normal	Follow-up	
Predicate		< 18.7	≥ 18.7	Totals:
Normal	< 29.2 N=118	114	4	118
Follow-Up	≥ 29.2 N = 15	0	15	15
	Totals:	114	19	133

The Accuwell 17-OHP EIA (ON Eluate) results for this population were: mean =12.2 ng/ml, with a range of 3.4 to 67.5 ng/ml; while the predicate kit yielded: mean =16.2 ng/ml, with a range of 6.9 to 81.0 ng/ml..

The correlation was found to be: y (Predicate) = 1.156 (Accuwell ON Eluate) + 2.030, R = 0.9729

Table 14: Predicate vs Accuwell 3 Hour Eluate
Values at the 95th Percentile for a
Population of Babies **≥2500 gm and 2-3 Days** of Age at Sample Collection

		95th Percentile		
	Accuwell	Normal	Follow-up	
Predicate		< 17.7	≥ 17.7	Totals:
Normal	< 29.2 N=118	115	3	118
Follow-Up	≥ 29.2 N = 15	0	15	15
	Totals:	115	18	133

The Accuwell 17-OHP EIA (3Hr Eluate) results for this population were: mean =12.8 ng/ml, with a range of 4.5 to 69.6 ng/ml; while the predicate kit yielded: mean =16.2 ng/ml, with a range of 6.9 to 81.0 ng/ml.

The correlation was found to be: y (Predicate) = 1.150 (Accuwell ON Direct) + 1.495, R = 0.9691

Table 15: Predicate vs Accuwell O/N Direct
Values at the 97.5th and 95th Percentiles for a
Population of **Babies ≥2500 gm and ≥ 4 Days** of Age at Sample Collection

		97.5th Percentile				95th Percentile			
	Accuwell	Normal	Follow-up			**Accuwell**	Normal	Follow-up	
Predicate		< 14.8	≥ 14.8	Totals:	**Predicate**		< 11.5	≥ 11.5	Totals:
Normal	< 21.9 N=192	181	11	192	Normal	< 21.9 N=192	170	22	192
Follow-Up	≥ 21.9 N = 5	0	5	5	Follow-Up	≥ 21.9 N = 5	0	5	5
	Totals:	181	16	197		Totals:	170	27	197

The Accuwell 17-OHP EIA (ON Direct) results for this population were: mean =7.3 ng/ml, with a range of 2.2 to 22.6 ng/ml; while the predicate kit yielded: mean =9.5 ng/ml, with a range of 2.7 to 31.0 ng/ml..

The correlation was found to be: y (Predicate) = 1.090 (Accuwell ON Direct) + 1.565, R = 0.9538

Table 16: Predicate vs Accuwell O/N Eluate
Values at the 97.5th and 95th Percentiles for a
Population of Babies **≥2500 gm and ≥ 4 Days** of Age at Sample Collection

		97.5th Percentile				95th Percentile			
	Accuwell	Normal	Follow-up			**Accuwell**	Normal	Follow-up	
Predicate		< 15.2	≥ 15.2	Totals:	**Predicate**		< 13.6	≥ 13.6	Totals:
Normal	< 21.9 N=192	181	11	192	Normal	< 21.9 N=192	178	14	192
Follow-Up	≥ 21.9 N = 5	1	4	5	Follow-Up	≥ 21.9 N = 5	0	5	5
	Totals:	182	15	197		Totals:	178	19	197

The Accuwell 17-OHP EIA (ON Eluate) results for this population were: mean =7.8 ng/ml, with a range of 1.4 to 26.5 ng/ml; while the predicate kit yielded: mean =9.5 ng/ml, with a range of 2.7 to 31.0 ng/ml..

The correlation was found to be: y (Predicate) = 1.070 (Accuwell ON Eluate) + 1.172, R = 0.9142

Table 17: Predicate vs Accuwell 3 Hour Eluate
Values at the 97.5th and 95th Percentiles for a
Population of **Babies ≥2500 gm and ≥ 4 Days** of Age at Sample Collection

		97.5th Percentile				95th Percentile			
	Accuwell	Normal	Follow-up			**Accuwell**	Normal	Follow-up	
Predicate		< 15.6	≥ 15.6	Totals:	**Predicate**		< 14.1	≥ 14.1	Totals:
Normal	< 21.9 N=192	176	16	192	Normal	< 21.9 N=192	174	18	192
Follow-Up	≥ 21.9 N = 5	0	5	5	Follow-Up	≥ 21.9 N = 5	0	5	5
	Totals:	176	21	197		Totals:	174	23	197

The Accuwell 17-OHP EIA (3Hr Eluate) results for this population were: mean =8.3 ng/ml, with a range of 2.2 to 28.4 ng/ml; while the predicate kit yielded: mean =9.5 ng/ml, with a range of 2.7 to 31.0 ng/ml..

The correlation was found to be: y (Predicate) = 1.024 (Accuwell 3Hr Eluate) + 1.081, R = 0.9350

Table 18: Predicate vs Accuwell O/N Direct
Values at the 99th and 97.5th Percentiles for a
Population of Babies **1400 - 2500 gm and 1-6 Days** of Age at Sample Collection

		99th (100th) Percentile					97.5th Percentile		
	Accuwell	Normal	Follow-up			**Accuwell**	Normal	Follow-up	
Auto DelFIA		< 46.0	≥ 46.0	Totals:	**Auto DelFIA**		< 43.9	≥ 43.9	Totals:
Normal	< 45.1 N=28	28	0	28	Normal	< 40.3 N=26	26	0	26
Follow-Up	≥ 45.1 N = 2	1	1	2	Follow-Up	≥ 40.3 N = 4	3	1	4
	Totals:	29	1	30		Totals:	29	1	30

The Accuwell 17-OHP EIA (ON Direct) results for this population were: mean =19.3 ng/ml, with a range of 6.5 to 47.8 ng/ml; while the predicate kit yielded: mean =25.5 ng/ml, with a range of 9.0 to 65.0 ng/ml..

The correlation was found to be: y (Predicate) = 1.22 (Accuwell ON Direct) + 1.971, R = 0.9556

Table 19: Predicate vs Accuwell O/N Eluate
Values at the 99th and 97.5th Percentiles for a
Population of Babies **1400 - 2500 gm and 1-6 Days** of Age at Sample Collection

		99th (100th) Percentile					97.5th Percentile		
	Accuwell	Normal	Follow-up			**Accuwell**	Normal	Follow-up	
Auto DelFIA		< 31.7	≥ 31.7	Totals:	**Auto DelFIA**		< 28.4	≥ 28.4	Totals:
Normal	< 45.1 N=28	24	4	28	Normal	< 40.3 N=26	23	3	26
Follow-Up	≥ 45.1 N = 2	1	1	2	Follow-Up	≥ 40.3 N = 4	1	3	4
	Totals:	25	5	30		Totals:	24	6	30

The Accuwell 17-OHP EIA (ON Eluate) results for this population were: mean =19.2 ng/ml, with a range of 6.7 to 40.2 ng/ml; while the predicate kit yielded: mean =25.5 ng/ml, with a range of 9.0 to 65.0 ng/ml..

The correlation was found to be: y (Predicate) = 1.234 (Accuwell ON Direct) + 1.812, R = 0.8770

Table 20: Predicate vs Accuwell 3Hr Eluate
Values at the 99th and 97.5th Percentiles for a
Population of Babies **1400 - 2500 gm and 1-6 Days** of Age at Sample Collection

		99th (100th) Percentile					97.5th Percentile		
	Accuwell	Normal	Follow-up			**Accuwell**	Normal	Follow-up	
Auto DelFIA		< 33.3	≥ 33.3	Totals:	**Auto DelFIA**		< 27.7	≥ 27.7	Totals:
Normal	< 45.1 N=28	26	2	28	Normal	< 40.3 N=26	23	3	26
Follow-Up	≥ 45.1 N = 2	1	1	2	Follow-Up	≥ 40.3 N = 4	2	2	4
	Totals:	27	3	30		Totals:	25	5	30

The Accuwell 17-OHP EIA (3Hr Eluate) results for this population were: mean =19.3 ng/ml, with a range of 6.6 to 39.2 ng/ml; while the predicate kit yielded: mean =25.5 ng/ml, with a range of 9.0 to 65.0 ng/ml..

The correlation was found to be: y (Predicate) = 1.174 (Accuwell 3Hr Eluate) + 2.795, R = 0.8421

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Neo-Genesis
c/o Jannet Baldwin
President,
Genesis Northwest, Inc.
15140 SE 82nd Drive
Clackamas, OR 97015

MAR 13 2007

Re: k060452
Trade Name: Accuwell 17-a-Hydroxyprogesterone Neonatal Screening Enzyme Immunoassay
Regulation Number: 21 CFR 862.1395
Regulation Name: 17-Hydroxyprogesterone test system.
Regulatory Class: Class I
Product Code: JLX
Dated: January 31, 2007
Received: February 2, 2007

Dear Ms. Baldwin:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at (240) 276-0490. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (240) 276-3150 or at its Internet address at http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Jean M. Cooper, M.S., D.V.M.

Jean M. Cooper, M.S., D.V.M.
Director
Division of Chemistry and Toxicology
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

Indications for Use

510(k) Number (if known):

k060452

Device Name:

Accuwell 17 a-Hydroxyprogesterone Neonatal Screening Enzyme Immunoassay

Indications For Use:

The Accuwell™ 17α-Hydroxyprogesterone EIA Kit is designed for the quantitative measurement of 17α-Hydroxyprogesterone (17-OHP) concentrations in neonatal blood samples that have been collected onto Whatman 903®specimen collection paper. The results are used to screen newborns for classical congenital adrenal hyperplasia (CAH).

Prescription Use ___X___ AND/OR Over-The-Counter Use _____
(Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Devices (OIVD)

Carol Benson

...ion Sign-Off

Office of In Vitro Diagnostic Device
Evaluation and Safety

K060452